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TREATMENT FOR INFLAMMATORY BOWEL DISEASE

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TREATMENT FOR INFLAMMATORY BOWEL DISEASE

This application is a continuation-in-part of U.S. Patent Application Serial No. 07/835,139 filed February 12, 1992 and of U.S. Patent Application Serial No. 08/284,603 filed August 11, 1994.

U.S. Patent Application Serial Nos. 07/835,139 filed February 12, 1992 and 08/284,603 filed August 11, 1994, and international patent application Serial No. PCT/US93/00924 filed February 2, 1993 are herein incorporated by reference.

FIELD OF THE INVENTION

The present invention relates to a treatment for inflammatory bowel disease (IBD). More particularly, this invention relates to the use of antibodies recognizing the integrin VLA-4 (very late antigen-4) in the treatment of IBD.

BACKGROUND OF THE INVENTION

Inflammatory bowel disease, or IBD, is a collective term encompassing ulcerative colitis and Crohn's disease (ileitis), which are chronic inflammatory disorders of the gastrointestinal tract. Ulcerative colitis is confined to the large intestine (colon) and rectum, and involves only the inner lining of the intestinal wall. Crohn's disease may affect any section of the gastrointestinal tract (i.e., mouth, esophagus, stomach, small intestine, large intestine, rectum and anus) and may involve all layers of the intestinal wall. Both diseases are characterized by abdominal pain and cramping, diarrhea, rectal bleeding and fever. The symptoms of these diseases are usually progressive, and sufferers typically experience periods of remission followed by severe flareups.

IBD affects an estimated two million people in the United States alone. Although IBD is not considered a fatal illness, prolonged disease can lead to severe malnutrition affecting growth or to the formation of abscesses or intestinal scar tissue, leading in turn to infection or bowel obstruction.

IBD has no cure, and the exact causes of IBD are not yet understood. Conventional treatments for IBD have involved anti-inflammatory drugs, immunosuppressive drugs

and surgery. Sulfasalazine and related drugs having the bioactive 5-amino-salicylic acid (5-ASA) moiety are widely used to control moderate IBD symptoms and to maintain remission. Severe inflammation is often treated with powerful corticosteroids and sometimes ACTH or with immunosuppressants such as 6-mercaptopurine and azathioprine. The most common surgical treatments for severe chronic IBD are intestinal resections and, ultimately, colectomy, which is a complete cure only for ulcerative colitis.

Severe side effects are associated with the drugs commonly prescribed for IBD, including nausea, dizziness, changes in blood chemistry (including anemia and leukopenia), skin rashes and drug dependence; and the surgical treatments are radical procedures that often profoundly alter the everyday life of the patient. Accordingly, there is a great need for treatments for IBD that are effective yet less severe in their side effects and are less invasive of the IBD sufferer's body and quality of life.

The search for the causes of IBD and more effective treatments has led several investigators to study diseased and normal tissue on a cellular level. This has led to observations of variations in the normal content of intestinal mucin (Podolsky, 1988 [1]) and to the observation of colonic glycoproteins that emerge only in diseased tissue (Podolsky and Fournier, 1988a [2], 1988b [3]). Researchers have observed that the cell adhesion molecule ICAM-1 is expressed at elevated levels in IBD tissue (Malizia et al., 1991 [4]). This molecule is thought to mediate leukocyte recruitment to sites of inflammation through adhesion to leukocyte surface ligands, i.e., LFA-1 (CD11a/CD18 complex) on all

leukocytes and Mac-1 (CD11b/CD18) on phagocytes. (See, e.g., Springer, 1990 [5].) Because flareups of IBD are often accompanied by increased concentrations of neutrophils and lymphocytes in the intestinal submucosa, blocking of interactions between endothelial cell receptors (such as ICAM-1) and their leukocyte ligands (such as LFA-1, Mac-1) has been proposed as a treatment for IBD.

Another cell adhesion molecule, VCAM-1 (vascular cell adhesion molecule-1) is expressed on inflamed endothelium and has been shown to recognize the $\alpha_4\beta_1$ integrin, VLA-4, expressed on the surface of all leukocytes except neutrophils (See, e.g., Springer, 1990 [5]; WO 92/00751 (Nielsen et al. [33]); and Weller et al., 1991 [34]). VCAM-1 also has been found to be expressed constitutively in noninflamed tissue, including Peyer's patch follicular dendritic cells (Freedman et al., 1990 [6]; Rice et al., 1991 [7]). Additionally, besides mediating cell adhesion events, VCAM-1 also has recently been determined to play a costimulatory role, through VLA-4, in T cell activation (Burkly et al., 1991 [8]; Damle and Arrufo, 1991 [9]; van Seventer et al., 1991 [10]). Accordingly, further study of VCAM-1 has been taken up to investigate whether it plays a role as a regulator of the immune response as well as a mediator of adhesion in vivo.

It has now been surprisingly discovered that administering anti-VLA-4 antibody significantly reduces acute inflammation in a primate model for IBD. Cotton top tamarins suffering from a spontaneous intestinal inflammation comparable to ulcerative colitis in humans that were treated with an anti-VLA-4 antibody (HP1/2) showed significant reduction in inflammation of biopsied intestinal tissue.

SUMMARY OF THE INVENTION

Accordingly, the present invention provides novel methods for the treatment of IBD and further provides new pharmaceutical compositions useful in the treatment of IBD. In particular, the present invention provides a method comprising the step of administering to an IBD sufferer an anti-VLA-4 antibody, such as antibody HP1/2 Also contemplated is the use of analogous antibodies, antibody fragments, soluble proteins and small molecules that mimic the action of anti-VLA-4 antibodies in the treatment of IBD.

DETAILED DESCRIPTION OF THE INVENTION

The technology for producing monoclonal antibodies is well known. Briefly, an immortal cell line (typically myeloma cells) is fused to lymphocytes (typically splenocytes) from a mammal immunized with whole cells expressing a given antigen, e.g., VLA-4, and the culture supernatants of the resulting hybridoma cells are screened for antibodies against the antigen. (See, generally, Kohler and Milstein, 1975 [11].)

Immunization may be accomplished using standard procedures. The unit dose and immunization regimen depend on the species of mammal immunized, its immune status, the body weight of the mammal, etc. Typically, the immunized mammals are bled and the serum from each blood sample is assayed for particular antibodies using appropriate screening assays. For example, anti-VLA-4 antibodies may be identified by immunoprecipitation of ^{125}I-labeled cell lysates from VLA-4-expressing cells. (See, Sanchez-Madrid et al., 1986 [13] and Hemler et al., 1987 [14].) Anti-VLA-4 antibodies may also be identified by flow cytometry, e.g., by measuring fluorescent staining of Ramos cells

incubated with an antibody believed to recognize VLA-4 (see, Elices et al., 1990 [15]). The lymphocytes used in the production of hybridoma cells typically are isolated from immunized mammals whose sera have already tested positive for the presence of anti-VLA-4 antibodies using such screening assays.

Typically, the immortal cell line (e.g., a myeloma cell line) is derived from the same mammalian species as the lymphocytes. Preferred immortal cell lines are mouse myeloma cell lines that are sensitive to culture medium containing hypoxanthine, aminopterin and thymidine ("HAT medium"). HAT-sensitive mouse myeloma cells may be fused to mouse splenocytes, e.g., using 1500 molecular weight polyethylene glycol ("PEG 1500"). Hybridoma cells resulting from the fusion are then selected using HAT medium, which kills unfused and unproductively fused myeloma cells (unfused splenocytes die after several days because they are not transformed). Hybridomas producing a desired antibody are detected by screening the hybridoma culture supernatants. For example, hybridomas prepared to produce anti-VLA-4 antibodies may be screened by testing the hybridoma culture supernatant for secreted antibodies having the ability to bind to a recombinant α_4-subunit-expressing cell line, such as transfected K-562 cells (see, Elices et al., [15]).

To produce anti VLA-4-antibodies, hybridoma cells that test positive in such screening assays may be cultured in a nutrient medium under conditions and for a time sufficient to allow the hybridoma cells to secrete the monoclonal antibodies into the culture medium. Tissue culture techniques and culture media suitable for hybridoma cells are well known. The conditioned hybridoma

culture supernatant may be collected and the anti-VLA-4 antibodies optionally further purified by well-known methods.

Alternatively, the desired antibody may be produced by injecting the hybridoma cells into the peritoneal cavity of a mouse primed with 2,6,10,14-tetramethylpentadecane (PRISTANE; Sigma Chemical Co., St. Louis MO). The hybridoma cells prcliferate in the peritoneal cavity, secreting the antibody, which accumulates as ascites fluid. The antibody may be harvested by withdrawing the ascites fluid from the peritoneal cavity with a syringe.

Several anti-VLA-4 monoclonal antibodies have been previously described (see, e.g., Sanchez-Madrid et al., 1986 [12]; Hemler et al. (1987) [13]; Pulido et al. (1991) [14]). For the experiments herein, an anti-VLA-4 monoclonal antibody designated HP1/2 (obtained from Biogen, Inc., Cambridge, MA) was used. The variable regions of the heavy and light chains of the anti-VLA-4 antibody HP1/2 have been cloned, sequenced and expressed in combination with constant regions of human immunoglobulin heavy and light chains. Such a chimeric HP1/2 antibody is similar in specificity and potency to the murine HP1/2 antibody, and may be useful in methods of treatment according to the present invention. Similarly, humanized recombinant anti-VLA-4 antibodies may be useful in these methods. The HP1/2 V_H DNA sequence and its translated amino acid sequences are set forth in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The HP1/2 V_K DNA sequence and its translated amino acid sequence are set forth in SEQ ID NO: 3 and SEQ ID NO: 4, respectively.

Monoclonal antibodies such as HP1/2 and other anti-VLA-4 antibodies (e.g., Mab HP2/1, HP2/4, L25, P4C2) capable of recognizing the α chain of VLA-4 will be useful in the present invention. It is most preferred that the antibodies will recognize the B1 or B2 epitopes of the VLA-α_4 chain (see, Pulido et al. (1991) [15]). While not wishing to be bound by one scientific theory, anti-VLA-4 antibodies used according to the method of the present invention may specifically inhibit, at least for an initial period, the migration of VLA-4-expressing leukocytes to inflamed sections of the gut. Or, the release of inflammatory mediators and cytokines by leukocytes already recruited to IBD tissue may be blocked by anti-VLA-4 antibodies that prevent some form of VCAM-1-mediated signal transduction, such as the T cell co-activation observed previously (e.g, Burkly et al. 1991 [8]). Monoclonal antibody HP1/2 has been shown to block leukocyte adhesion to VCAM-1-expressing cells but not to promote VLA-4-mediated T cell activation.

The method of the present invention comprises administering to a mammal suffering from inflammatory bowel disease a composition comprising an anti-VLA-4 antibody. The examples below set forth the results observed in cotton top tamarins. The physiological and histochemical similarities between a spontaneous chronic diffuse colitis observed in the cotton top tamarin (CTT) and IBD humans has been documented (see, e.g., Podolsky et al., 1985a [16], Podolsky et al., 1985b [17]). Prior studies have also demonstrated parallel responses in CTTs to therapeutic compounds used in the management of the human IBD (see, e.g., Madara et al., 1985 [18]). Accordingly, the results reported herein will be relevant

and applicable to, and the method claimed will be useful in any mammal, including humans, suffering from IBD.

The anti-VLA-4 antibody administered in accordance with the present invention may be administered prophylactically to a chronic IBD sufferer, to bring about or maintain remission of the disease; however, preferably the method of the present invention is used to treat acute flareups of the disease.

The anti-VLA-4 antibody can be administered in the form of a composition comprising an anti-VLA-4 antibody and a pharmaceutically acceptable carrier. Preferably, the composition will be in a form suitable for intravenous injection. For acute flareups of ulcerative colitis or Crohn's disease, dosages of from 0.05 mg/kg-patient/day to 5.0 mg/kg-patient/day (preferably from 0.5 mg/kg-patient/day to 2.0 mg/kg-patient/day) may be used, although higher or lower dosages may be indicated with consideration to the age, sensitivity, tolerance, and other characteristics of the patient, the acuteness of the flareup, the history and course of the disease, plasma level and half-life of the antibody employed and its affinity for its recognition site, and other similar factors routinely considered by an attending physician. For maintenance of remission from active disease, dosages from 0.05 mg/kg-patient/day to 5.0 mg/kg-patient/day (preferably from 0.5 mg/kg-patient/day to 2.0 mg/kg-patient/day) may be used, although higher or lower dosages may be indicated and employed with advantageous effects considering the age, sensitivity, tolerance, and other characteristics of the patient, the pattern of flareups, the history and course of the disease, the plasma level and half-life of the antibody employed and its affinity for its recognition site, and other similar factors

routinely considered by an attending physician. Dosages may be adjusted, for example, to provide a particular plasma level of antibody, e.g., in the range of 5-30 μg/ml, more preferably 10-15 μg/ml, for murine antibodies, and to maintain that level, e.g., for a period of time (e.g., 1 week) or until clinical results are achieved (e.g., flareup subsides). Chimeric and humanized antibodies, which would be expected to be cleared more slowly, will require lower dosages to maintain an effective plasma level. Also, antibodies or fragments having high affinity for VLA-4 will need to be administered less frequently or in lower doses than antibodies or antibody fragments of lesser affinity.

Suitable pharmaceutical carriers include, e.g., sterile saline, physiological buffer solutions and the like. The pharmaceutical compositions may additionally be formulated to control the release of the active ingredients or prolong their presence in the patient's system. Numerous suitable drug delivery systems are known for this purpose and include, e.g., hydrogels, hydroxmethylcellulose, microcapsules, liposomes, microemulsions, microspheres, and the like. Phosphate buffered saline (PBS) is a preferred carrier for injectible compositions.

It will also be recognized that for the purposes of the present invention, antibodies capable of binding to the α_4 subunit of VLA-4 must be employed. It is preferred that monoclonal antibodies be used.

In addition to naturally produced antibodies, suitable recombinant antibodies capable of binding to VLA-4 may alternatively be used. Such recombinant antibodies include antibodies produced via recombinant DNA

techniques, e.g., by transforming a host cell with a suitable expression vector containing DNA encoding the light and heavy immunoglobulin chains of the desired antibody, and recombinant chimeric antibodies, wherein some or all of the hinge and constant regions of the heavy and/or the light chain of the anti-VLA-4 antibody have been substituted with corresponding regions of an immunoglobulin light or heavy chain of a different species (i.e., preferably the same species as the IBD sufferer being treated, to minimize immune response to the administered antibody). (See, e.g., Jones et al., 1986 [19], Ward et al., 1989 [20], and U.S. Patent 4,816,397 (Boss et al.) [21], all incorporated herein by reference.) Recombinant antibodies specifically contemplated herein include CDR-grafted antibodies or "humanized" antibodies, wherein the hypervariable regions of, e.g., murine antibodies are grafted onto framework regions of, e.g., a human antibody. (See, e.g., Riechmann et al., 1988 [22]; Man Sung Co et al., 1991 [23]; Brown, Jr., 1991 [24].)

Furthermore, VLA-4-binding fragments of anti-VLA-4 antibodies, such as Fab, Fab', $F(ab')_2$, and F(v) fragments; heavy chain monomers or dimers; light chain monomers or dimers; and dimers consisting of one heavy chain and one light chain are also contemplated herein. Such antibody fragments may be produced by chemical methods, e.g., by cleaving an intact antibody with a protease, such as pepsin or papain, or via recombinant DNA techniques, e.g., by using host cells transformed with truncated heavy and/or light chain genes. Heavy and light chain monomers may similarly be produced by treating an intact antibody with a reducing agent such as dithiothreitol or β-mercaptoethanol or by using host cells

transformed with DNA encoding either the desired heavy chain or light chain or both.

As an alternative to hybridoma technology, antibody fragments having the desired anti-VLA-4 specificities may be isolated by phage cloning methods. (See, e.g., Clackson et al., 1991 [25].)

Also, from the foregoing discussion it will be apparent that other polypeptides and molecules which bind to VLA-4 with sufficient specificity to inhibit VLA-4/VCAM-1 interactions or to inhibit transduction of VCAM-1-mediated signaling will be effective in the treatment of IBD in the same manner as anti-VLA-4 antibodies. For example, a soluble form of VCAM-1 (see, e.g., Osborn et al. 1989 [26]) or a fragment thereof may be administered to compete for the VLA-4 binding site, thereby leading to effects similar to the administration of anti-VLA-4 antibodies. Small molecules that mimic the binding domain of a VLA-4 ligand and fit the receptor domain of VLA-4 may also be employed. (See also, Devlin et al., 1990 [27], Scott and Smith, 1990 [28], and U.S. Patent 4,833,092 (Geysen) [29], all incorporated herein by reference.) The use of such VLA-4-binding polypeptides or molecules that effectively decrease inflammation in IBD tissue in treated subjects is contemplated herein as an alternative method for treatment of IBD.

It is also contemplated that anti-VLA-4 antibodies may be used in combination with other antibodies having a therapeutic effect on IBD. For instance, to the extent that the beneficial effects reported herein are due to the inhibition of leukocyte recruitment to endothelium, combinations of anti-VLA-4 antibodies with other antibodies that interfere with the adhesion between leukocyte antigens and endothelial cell

receptor molecules may be advantageous. For example, in addition to the use of anti-VLA-4 antibodies in accordance with this invention, the use of anti-ELAM-1 antibodies, anti-VCAM-1 antibodies, anti-ICAM-1 antibodies, anti-CDX antibodies, anti-CD18 antibodies, and/or anti-LFA-1 antibodies may be advantageous.

When formulated in the appropriate vehicle, the pharmaceutical compositions contemplated herein may be administered by any suitable means such as orally, intraesophageally or intranasally, as well as subcutaneously, intramuscularly, intravenously, intra-arterially, or parenterally. Ordinarily intravenous (i.v.) or parenteral administration will be preferred to treat flareup conditions; oral administration in a timed release vehicle will be preferred to maintain remission.

Improvement for IBD patients as a result of the methods of this invention can be evaluated by any of a number of methods known to practitioners in this art. For example, improvement in observed symptomology such as the Truelove-Witts criteria (see, e.g., Lichtiger, et al., 1990 [30]) may be used, or specimens of colon tissue may be biopsied and characterized histologically (see, e.g., Madara et al., 1985 [18]).

The methods and compositions of the present invention will be further illuminated by reference to the following examples, which are presented by way of illustration and not of limitation.

EXAMPLE I

VCAM1 Expression in the Colon

Experiments were performed to determine whether active IBD involved the expression of endothelial cell surface proteins involved in leukocyte adhesion. Expression of VCAM-1 in colon tissue of IBD sufferers and

normal or uninvolved colon tissue controls was evaluated. Human colonoscopic biopsy tissue samples were obtained, with informed consent, and prepared as frozen sections by mounting in OCT compound (TissueTek) and quick freezing in isopentane/liquid nitrogen. The human colon samples were from normal colon, active ulcerative colitis colon (UC-active), inactive ulcerative colitis colon (UC-inactive), uninvolved ulcerative colitis colon (UC-uninvolved), active Crohn's Disease colon (CD-active), and uninvolved Crohn's Disease colon (CD-uninvolved).

Frozen sections (~4μ) were placed on gelatin-coated slides (1% gelatin, heated at 60° C for 1-2 min., air dried, 1% formaldehyde at room temp., air dried), air dried 30 minutes, fixed in acetone for ten minutes at 4° C, washed three times in PBS and treated with 0.3% H_2O_2 in methanol (30 min., room temp.). The slides were then washed with PBS for 30 minutes, incubated with dilute normal human serum (1:100), and incubated with anti-VCAM-1 antibody 4B9 (1:100; obtained as a gift from Dr. John Harlan) for 60 minutes at room temperature. Control slides were incubated with an anti-bovine serum albumin (anti-BSA) antibody (Sigma Chemical Co., St. Louis MO). The samples were then washed with PBS for 10 minutes and incubated with a secondary biotinylated rabbit anti-mouse immunoglobulin (Dako Corp., Santa Barbara, CA) for 60 minutes at room temperature, then visualized using avidin-linked peroxidase (VECTASTAIN, Vector Labs, Burlingame CA).

The results of these tests are set forth in the following TABLE I:

TABLE I

Endothelial Cell Staining In Human Tissue

Tissue (n)	VCAM-1 Expression n	(%)
Normal (11)	6	(54.4)
UC active (23)	14	(60.9)
UC inactive (8)	5	(62.5)
UC uninvolved (10)	4	(40.0)
CD active (9)	5	(55.5)
CD uninvolved (12)	7	(58.3)

These data confirm the observations such as those reported by Freedman et al. [6] and Rice et al. [7] that VCAM-1 is expressed in both IBD-involved colon tissue and in normal colon tissue. In both CD and UC tissues, VCAM-1 was observed by immunocytochemistry in about 60% of samples.

EXAMPLE II

Anti-VLA-4 Antibody Recognition of CTT White Blood Cells

An anti-VLA-4 monoclonal antibody (HP1/2, obtained from Biogen, Inc., Cambridge MA) was tested to confirm that it recognized an epitope on CTT leukocytes.

Blood samples (3 ml) from CTTs were heparinized and the CTT peripheral blood mononuclear leukocytes (PBLs) were isolated using a Ficoll-Hypaque gradient (Pharmacia) according to the manufacturer's instructions for isolation of human PBLs. CTT PBLs were examined for their ability to bind to the murine anti-human VLA-4 monoclonal antibodies HP1/2 and HP2/1 by FACS analysis using a Becton Dickenson FACStar and standard techniques (see, e.g., Lobb et al., 1991a [31]). Both monoclonal antibodies bound to

CTT PBLs, indicating that both human and CTT VLA4 have similar epitopes recognized by these two antibodies.

CTT PBLs were also observed to adhere to microtiter plates coated with immobilized recombinant soluble human VCAM-1 (Biogen, Inc.), which binding was blocked by HP1/2 and HP2/1. These results show that CTT PBLs bind to VCAM-1 in a VLA-4-dependent manner, and that HP1/2 and HP2/1 block the interaction of CTT VLA-4 with human VCAM-1. (Cf. Lobb et al., 1991b [32].)

EXAMPLE III

Cotton Top Tamarin Trials

A stock solution in sterile saline of the anti-VLA-4 antibody, HP1/2 (IgG1), and a placebo control (saline only), were prepared for administration to ten cotton top tamarins (CTTs) exhibiting symptoms of spontaneous colitis (i.e., diarrhea, etc.; see, Madara et al. [18]). Five CTTs received HP1/2 and five received placebo, by intravenous injection. The CTTs receiving HP1/2 were injected with 1 mg HP1/2 per day (i.e., about 2 mg/kg/day, based on approximate half-kilogram weight of a CTT) for eight days (on Days 0, 1, 2, 3, 4, 5, 6, and 7 of the trial). Colon tissue samples obtained from the animals were biopsied every other day (on Days 0, 2, 4, 6, 8, and 10 of the trial).

Data from the biopsies were used to determine an acute inflammation index for each animal, giving a semi-quantitative analysis of the course of the colitis. (See, Madara et al. [18].) The inflammation indices before the trial began (Day 0) and at the end of the trial at Day 10 are set forth in Table II, below: ("Treated CTTs" received antibody HP1/2; "Control CTTs" received placebo)

TABLE II

	Day 0	Day 10
Treated CTTs	AII*	AII
1	2	0
2	1	0
3	1	0
4	2	0
5	2	1
MEAN	1.6	0.2
Control CTTs		
C1	2	0
C2	2	1
C3	1	1
C4	2	2
C5	2	2
MEAN	1.8	1.2

* AII = acute inflammation index

These results show that treatment with anti-VLA-4 antibody resulted in a significant ($p < 0.01$) decrease in acute inflammation index.

EXAMPLE IV

The trial described in Example III was repeated using 14 CTTs, 7 receiving HP1/2 and 7 receiving placebo. The change in acute inflammation index from Day 0 to Day 10 is set forth in Table III:

TABLE III

	Day 0	Day 10
Treated CTTs	AII	AII
6	2	0
7	2	0
8	2	0
9	2	0
10	2	0
11	2	1
12	2	2
MEAN	2.0	0.43
Control CTTs		
C6	2	2
C7	2	2
C8	1	1
C9	2	1
C10	2	1
C11	2	0
C12	1	0
MEAN	1.71	1.00

The foregoing results show a significant decrease in acute inflammation in the CTTs receiving HP1/2.

The foregoing examples are intended as an illustration of the method of the present invention and are not presented as a limitation of the invention as claimed hereinafter. From the foregoing disclosure, numerous modifications and additional embodiments of the invention will be apparent to those experienced in this art. For example, actual dosage used, the type of antibody, antibody fragment or analog used, mode of administration, exact composition, time and manner of

administration of the treatment, and many other features all may be varied without departing from the above description. All such modifications and additional embodiments are within the contemplation of this application and within the scope of the appended claims.

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The foregoing documents are incorporated herein by reference.